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(71)(72) Applicant and Inventor: PRENDERGAST, Patrick, T. [IE/IE]; Baybush, Straffen, County Kildare (IE).

(74) Agent: BARDEHLE PAGENBERG DOST ALTENBURG FROHWITTER GEISSLER & PARTNER; Postfach 86 06 20, D-81633 München (DE).

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(54) Title: COMBINATION THERAPY UTILISING 17-KETOSTEROIDS AND INTERLEUKIN INHIBITORS, OR INTERLEUKIN-10 POTIONALLY WITH INTERLEUKIN INHIBITORS

(57) Abstract

There are provided medicaments, methods of making them, and kits, which include (1) a 17-ketosteroid compound and/or (2) an Interleukin-10 inhibitor, an Interleukin-12 inhibitor, an Interleukin-2 inhibitor, alpha-fetoprotein, or a lysosomotropic agent. Also provided are methods of treating a patient with Interleukin-10, or with Interleukin-12 and an Interleukin-10 inhibitor. There are also provided methods of treatment involving such compounds or combinations of compounds, including enhancing the T_H1 immune protective response when using the 17-ketosteroid compound as an anti-viral, anti-bacterial, anti-mycoplasma or anti-intra cellular parasitic agent, and other treatments for various compounds and combinations as described.

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COMBINATION THERAPY UTILISING 17-KETOSTEROIDS AND INTERLEUKIN INHIBITORS, OR INTERLEUKIN-10 POTIONALLY WITH INTERLEUKIN INHIBITORS

BACKGROUND OF THE INVENTION

Anti-viral agents which inhibit replication of viruses have been known since the mid 1960's. Several hundred or more of these agents are now known, including those of general Formula I which are discussed in U.S. Patent No. 4,956,355.

The compound N^6-(Δ^2-isopentenyl) adenosine (IPA), which is of general formula II, illustrated below, has been used previously in clinical trials involving the treatment of cancer. (CYTOKININS AS CHEMOTHERAPEUTIC AGENTS, Annals of the New York Academy of Science, 25, 225-234 Mittleman, Arnold et al. (1975)). IPA is a naturally occurring compound. For example, it has been shown to be an anticodon-adjacent nucleoside in certain t-RNAs (Biochimica et Biophysica Acta, 281:488-500. Gallo, Robert D., et al. (1972). IPA has been shown to have cytokinin properties, to inhibit the growth of human leukemic myoblasts, to inhibit the growth of cultured lymphocytes stimulated by phytohemagglutinin (PHA) at certain concentrations, and to stimulate the growth of cultured lymphocytes stimulated by PHA at lower concentrations (Gallo, et al.). Further, IPA has been used in clinical experiments on humans as a chemotherapeutic agent.

SUMMARY OF THE INVENTION

A method of enhancing the T_H1 immune response in a patient is disclosed, comprising administering to the patient a) an effective amount of a 17-ketosteroid compound and an effective amount of an Interleukin-10 inhibitor. The Interleukin-10 inhibitor can be antiserum to Interleukin-10, a compound effective for inhibiting synthesis or biological function of Interleukin-10 or an Interleukin-10 receptor molecule blocking agent. The Interleukin-10 inhibitor can also be canavanine sulfate, L-canavanine sulfate, herbimycin A, genistein, secalonic acid D, an isoflavonoid, a cytokinin, or an amphiphilic triterpenoid. In addition, the Interleukin-10 inhibitor can be selected from the group having the general formula as shown in Formula I herein.

A method of enhancing the T_H2 immune response in a patient is disclosed. The method comprises administering to the patient an effective amount of a 17-ketosteroid compound and an effective amount of an Interleukin-12 inhibitor. The Interleukin-12 inhibitor can be antiserum to Interleukin-12, a compound effective for inhibiting synthesis or biological function of Interleukin-12 or an Interleukin-12 receptor molecule blocking agent. The method can be used to treat a patient, e.g., for a bacterial infection or autoimmune condition such as lupus or graft versus host disease.

Compositions and pharmaceutical formulations useful for a combination therapy include a 17-ketosteroid compound and another compound as disclosed herein, e.g.,

an Interleukin-10 inhibitor, an Interleukin-2 inhibitor or an Interleukin-12 inhibitor. Articles of manufacture are also disclosed, comprising for example, packaging material, at least one unit-dosage of a 17-ketosteroid compound; and a label or package insert indicating that the 17-ketosteroid compound can be used in a method disclosed herein.

A method of treating a patient for an autoimmune condition comprises administering to the patient an effective amount of Interleukin-10. The method is useful for treating the patient for lupus, multiple sclerosis, graft versus host disease or as an adjuvant in vaccine therapy.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

According to the invention, there are provided combinations of compounds for use in restoring normal levels of Interleukin-12 (IL-12) and/or Interleukin-10 (IL-10) (cytokine inhibitory factor), by enhancing or curtailing the synthesis or effect of IL-12 and/or IL-10.

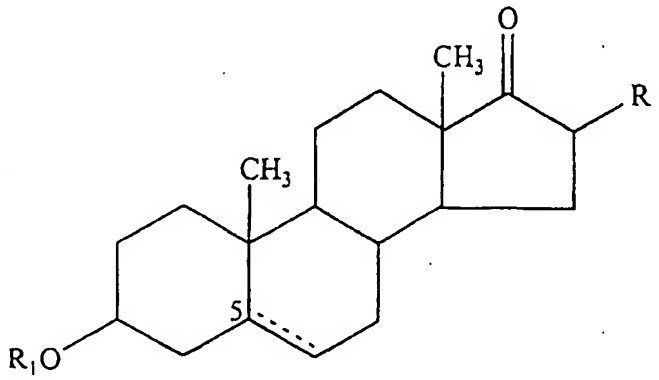
In one aspect of the present invention, the anti-viral agents (general Formula I herein) as disclosed in U.S. Patent No. 4,956,355 (Prendergast) have additional beneficial therapeutic effects when used in a combination therapy with agents that inhibit IL-10 synthesis and/or action, termed IL-10 inhibitory compounds or IL-10 inhibitors.

In another aspect of the present invention, there is provided a method of enhancing the T_H1 immune

protective response when using a 17-ketosteroid compound as an anti-viral, anti-bacterial, anti-mycoplasma, or anti-intracellular parasitic agent. An immune response for a given patient is enhanced for a T_H1 immune response (cell-mediated immunity) when T_H1 cytokines are produced. T_H1 cytokines can include but are not limited to gamma-interferon (γ-IFN), IL-2, and IL-12. A T_H1 immune protective response is required, for example, by patients in need of therapy for cancer, metastatic cancer, multi-drug resistant cancer, viral infection, parasite infection, multi-drug resistant bacterial infection and bacterial infection.

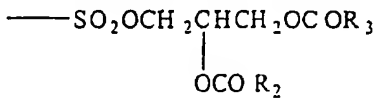
In one aspect, the novel method comprises the administration of a 17-ketosteroid compound and an IL-10 inhibitor to a patient. For the purposes of this invention, a patient is a mammal, e.g., a rat, a mouse, a human, a cow, or a dog. In preferred embodiments, a patient is a human. A patient can be in need of treatment for a disease or can be suspected of being in need of such treatment.

A 17-ketosteroid compound according to the invention has the following general formula I:



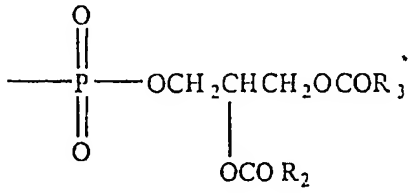
The broken line represents an optical double bond and the hydrogen atom at position 5 is present in the α- or β-configuration. The compound can also comprise a mixture of both configurations. R represents a hydrogen atom and R_1 represents, without limitation, a hydrogen atom or an SO_2OM group with M representing, without limitation, a hydrogen atom; a sodium atom; a sulphatide group; a phosphatide group; or a glucuronide group.

The sulphatide group can be the following:



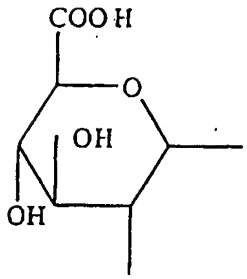
with R_2 and R_3, which may be the same or different, being straight or branched chain alkyl radicals of 1 to 14 carbon atoms.

The phosphatide group can be the following:



with R_2 and R_3, which may be the same or different, being straight or branched chain alkyl radical of 1 to 14 carbon atoms.

The glucuronide group can be the following:



When R_1 is other than a hydrogen atom, the compounds are conjugated compounds. Compounds according to general formula I are disclosed in U.S. Patent No. 4,956,355.

Preferably in the compound of formula I, R and R_1 are each hydrogen. An especially preferred compound is dehydroepiandrosterone (DHEA) wherein R and R_1 are each hydrogen and the double bond is present.

In a further embodiment of the invention, the compound is epiandrosterone wherein R and R_1 are each hydrogen and the double bond is absent (i.e., where the dotted line is shown in formula I, there is a single bond). This unsaturated 5-position steroid can also be prepared as an anti-viral agent wherein the R position is occupied by any of the following halogens: bromine, chlorine, fluorine, or iodines.

In a further embodiment of the invention, the compound is 16α- bromoepiandrosterone, wherein R is Br, R_1 is H, and the double bond is present. In a still further embodiment of the invention, the compound is according to

formula I, wherein R is Br, R_1 is H, and the double bond is not present.

Other preferred compounds are dehydroepiandrosterone sulphate, wherein R is H, R_1 is SO_2-OM (M is as hereinbefore defined), and the double bond is present, as well as 5β-androstan-3β-ol-17-one.

Alternatively, the compound is selected from dehydroepiandrosterone sulphatides, phosphatides, or glucuronide wherein R is H, R_1 is a sulphatide, phosphatide, or glucuronide group as hereinabove defined, and the double bond is present. In particular, when R_1 is not hydrogen, the compounds are DHEA conjugates such as hexyl sulfate, dodecyl sulfate, octadecyl sulfate, octadecanoylglycol sulfate, O-dihexadecylglycerol sulfate, hexadecane sulfonate, dioctadecanoylglycerol phosphate, or O-hexadecylglycerol phosphate.

The above-mentioned 17-ketosteroids can exist in a polymorph form.

An IL-10 inhibitor can be a compound that inhibits IL-10 activity, for example, antiserum to IL-10. Antiserum to IL-10 can be polyclonal, or monoclonal in origin. Antiserum to IL-10 can comprise intact antibody or can comprise antibody fragments that retain IL-10 binding specificity. Anti-IL-10 antibodies can be directed against IL-10 or peptide fragments possessing epitopes useful in inhibiting IL-10 activity *in vivo*. Further, IL-10 activity can be inhibited by soluble IL-10 receptor, for example, administered at about 20

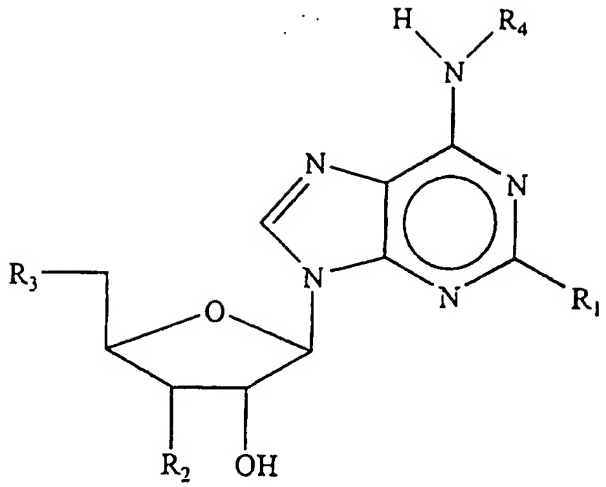
micrograms per day to about 200 micrograms per day by intravenous injection.

An IL-10 inhibitor can also be a compound that inhibits the synthesis of IL-10 *in vivo*. The synthesis of IL-10 can be inhibited by any of a variety of compounds, including one or a combination of the following compounds: canavanine sulfate, L-canavanine sulfate, herbimycin A (Wako Pure Chemicals Industries, Ltd., Japan), genistein (Sigma Chemicals Co., St. Louis, Mo., USA), secalonic acid D, isoflavonoids, cytokinins, amphiphilic triterpenoids, or analogues to the above.

An IL-10 inhibitor can also be a compound that blocks the IL-10 receptor in cells of a patient. For example, such compounds can be antibodies against the IL-10 receptor, anti-idiotype antibodies against an anti-IL-10 antibody, or antibodies against a portion of IL-10 that binds to the IL-10 receptor.

A compound that inhibits IL-10 can be identified, for example, by identifying compounds that have the ability to inhibit cyclic AMP activity. In addition, agents that demonstrate IL-10 inhibition as determined by standard assay methods can also serve as IL-10 inhibitors. Compounds that inhibit IL-10 synthesis can be identified, for example, by an IL-10 specific ELISA. Such compounds also include those that specifically alter transcription of IL-10 DNA, translation of IL-10 RNA, or processing of IL-10 protein.

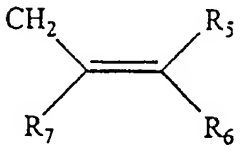
Suitable IL-10 inhibitors also include compounds of the following general formula II:



wherein:

R_1 = H, R_2 = CH_3, R_3 = CH_3 and R_4 = H, or

R_1 = H or CH_3S and R_4 =



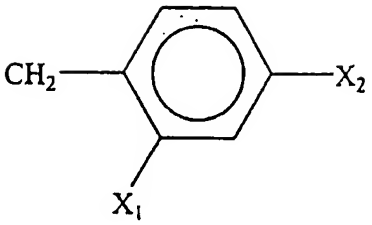
and

R_5 = CH_3, Cl, OH, or a monophosphate group

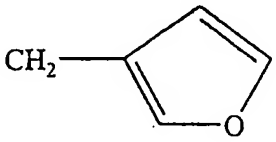
R_6 = CH_3, CH_2OH, or Cl

R_7 = H or Br

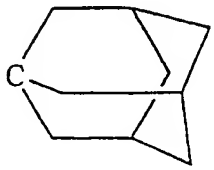
or R_1 = H and R_4 =



and X_1 and X_2 are independently selected from H, methyl, ethyl, hydroxyl, the halogens and carboxyl or R_4 =

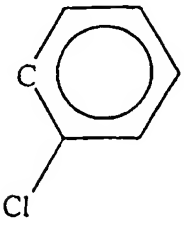


or R_4 =



or R_4 =

and R_8 =

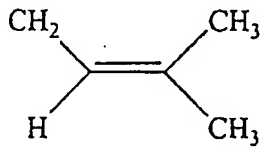


or $R_8 = (CH_2)_7CH_3$;

and R_2 and R_3 are linked to form a 3', 5'-cyclic monophosphate derivative, or a physiologically acceptable salt of any such compound. "Formula II" is used herein to refer to all such compounds and salts.

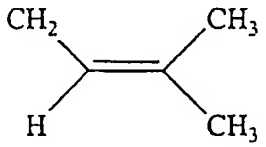
Listed below are chemical groups R_1-R_4 for especially preferred compounds IIa-IIu according to this invention.

IIa: R_1 = H, R_2 = OH, R_3 = OH and R_4 =



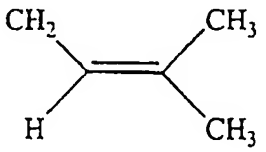
N^6 = (Δ^2-isopentenyl) adenosine

IIb: R_1 = R_2 = OH, R_3 = monophosphate, and R_4 =



N^6 = (Δ^2-isopentenyl) adenosine-5'-monophosphate

IIc: R_1 = H, R_2 and R_3 are linked to form a 3', 5'-cyclic monophosphate derivative, and R_4 =



N^6 = (Δ^2-isopentenyl) adenosine-3', 5'-cyclic monophosphate

IId: R_1 = H, R_2 = OH, R_3 = OH, and R_4 = $CH_2C_6H_6$

N^6 =benzyladenosine

IIe: R_1 = H, R_2 = OH

R_3 = R_3 = monophosphate, and R_4 = $CH_2C_6H_6$

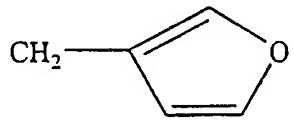
N^6-benzyladenosine-5'-monophosphate

IIf: R_1 = H, R_2 and R_3 are linked to form a 3', 5-cyclic monophosphate derivative and

R_4= $CH_2C_6H_6$

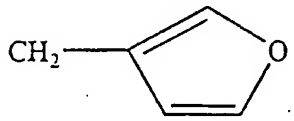
N^6-benzyladenosine-3', 5'-cyclic monophosphate

IIg: R_1 = H, R_2 = OH, R_3 = OH, and R_4 =



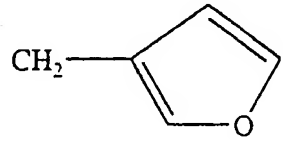
Furfuryladenosine

IIh: R_1 = H, R_2 = OH, R_3 = monophosphate and R_4 =



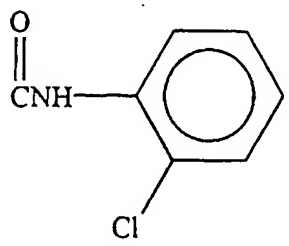
N^6-furfuryladenosine-5'-monophosphate

IIi: R_1 = H, R_2 and R_3 are linked to form a 3', 5'-cyclic monophosphate derivative, and R_4 =



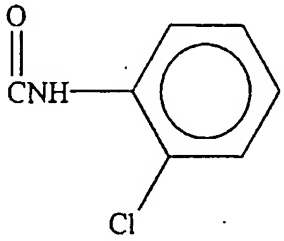
N^6-furfuryladenosine-3', 5'-cyclic monophosphate

IIj: R_1 = H, R_2 = OH, R_3 = OH and R_4 =



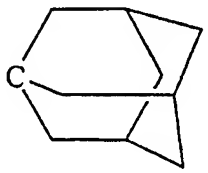
N-(purin-6-ylcarbamoyl)-O-chloroaniline ribonucleoside

IIk: R_1 = H, R_2 = OH, R_3 = monophosphate, and R_4 =



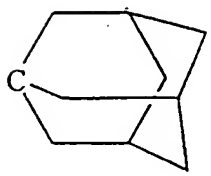
N-(purin-6-ylcarbamoyl)-O-chloroaniline ribonucleoside-5'-monophosphate

IIl: R_1 = H, R_2 = OH, R_3 = OH and R_4 =



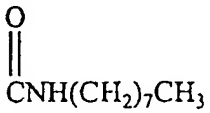
N^6-adamantyladenosine

IIm: R_1 = H, R_2 = OH, R_3 = monophosphate and R_4 =



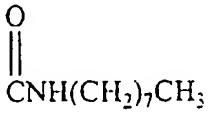
N^6-adamantyladenosine-5'-monophosphate

IIn: R_1 = H, R_2 = OH R_3 = OH and R_4 =



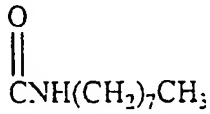
N-(purin-6-ylcarbamoyl)-n-octylamine ribonucleoside

IIo: R_1 = H, R_2 = OH, R_3 = monophosphate and R_4 =



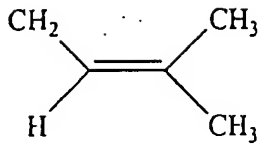
N-(purin-6-ylcarbamoyl)-n-octylamine ribonucleoside-5'-monophosphate

IIp: R_1 = H, R_2 and R_3 are linked to form a 3', 5'-cyclic monophosphate derivative, and R_4 =



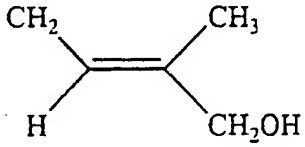
N-(purin-6-ylcarbamoyl)-n-octylamine ribonucleoside-3', 5'-cyclic monophosphate

IIq: R_1 = CH_3S, R_2 = OH, R_3 = OH and R_4 =



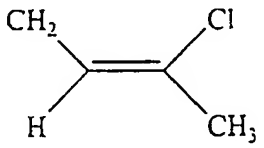
N-(Δ^2-isopentenyl)-2-methylthioadenosine

IIr: R_1 = H, R_2 = OH, R_3 = OH and R_4 =



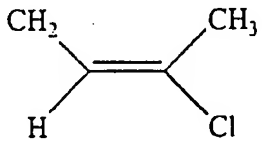
N^6-(4-hydroxy-3-methyl-trans-2-butenyl)-adenosine

IIs: R_1 = H, R_2 = OH, R_3 = OH, and R_4 =



N^6-(3-chloro-trans-2-butenyl) adenosine

IIt: R_1 = H, R_2 = OH, R_3 = OH and R_4 =



N^6-(3-chloro-cis-2-butenyl) adenosine

IIu: R_1 = H, R_2 = CH_3, R_3 = CH_3 and R_4 = H

Also included in the invention are one or more metabolites of the family of compounds of Formula II. Illustrative examples of metabolites include: N^6(Δ^2-

isopentenyl) adenine, 6-N-(3-methyl-3-hydroxybutylamino) purine, adenine, hypoxanthine, uric acid, and methylated xanthines.

A 17-ketosteroid compound and a IL-10 inhibitor can be administered essentially simultaneously, e.g., administration of each compound a few minutes or a few hours apart, or can be administered sequentially, e.g., several days apart, or more than a week apart. For example, administration of an IL-10 inhibitor can be followed by administration of a 17-ketosteroid. All such variations in administration of the combination therapy are encompassed within the scope of the invention.

The invention also includes the use of a 17-ketosteroid and an IL-10 inhibitor in the manufacture of a medicament for use in the treatment of a condition such as cancer, metastatic cancer, multi-drug resistant cancer, viral infection, parasite infection, multi-drug resistant bacterial infection and bacterial infection. It should be appreciated that more than one 17-ketosteroid and/or more than one IL-10 inhibitor may be used in some embodiments of the invention.

Compounds used according to this invention are administered by any suitable route, including enteral, parenteral, topical, oral, rectal, nasal, or vaginal routes. Parenteral routes include subcutaneous, intramuscular, intravenous, and sublingual administration. Topical routes include buccal and sublingual administration. In addition, the 17-ketosteroid compound and the IL-10 inhibitor can be

administered by the same route or by different routes, recognizing that the route by which a compound is administered depends upon the nature of the compound. For example, both compounds can be administered by injection or both compounds can be administered by mouth. As an alternative one compound can be administered by injection and the other compound by mouth.

Pharmaceutical formulations prepared according to the invention can include a 17-ketosteroid compound and an IL-10 inhibitor contained in macrophage-specific liposome micells of suitable size to facilitate phagocytosis, tablets (including coated tablets), elixirs, suspensions, syrups, inhalations, gelatine capsules in tablet form, dragees, syrups, suspensions, topical creams, suppositories, injectable solutions (such as a pharmaceutically acceptable solution which may include a carrier), or kits for the preparation of a syrup, suspension, topical cream, suppository or injectable solution just prior to use. Also, a 17-ketosteroid and an IL-10 inhibitor may be included in a composite which facilitates its slow release into the blood stream, e.g., a silicone disc, polymer beads or a transdermal patch.

If desired, pharmaceutical preparations prepared according to the invention can utilize conventional excipients, that is, pharmaceutically acceptable organic or inorganic carrier substances which do not deleteriously react with the compounds. Suitable pharmaceutically acceptable carriers include but are not

limited to water, salt solutions, alcohols, gum arabic, vegetable oils, gelatine, carbohydrates, magnesium stearate, talc, silicic-acid, viscous paraffin, fatty acid mono- and di-glycerides.

The preparative procedure may include the sterilization of the pharmaceutical preparations. The compounds may be mixed with auxiliary agents such as lubricants, preservatives, stabilizers, salts for influencing osmotic pressure, and the like, which do not react deleteriously with the compounds.

Compounds of Formula II have especially low toxicity for children and acceptable toxicity for adult humans, although it is somewhat higher than for children. IPA and IPA-like compounds can be stored dry almost indefinitely if protected from light and stored at -75°C. IPA is photosensitive and deteriorates at room temperature, whether in a solid form or in aqueous or ethanolic solutions. It has been found that the breakdown rate of IPA is approximately 3% per month in a dark container at room temperature.

This invention includes the use of physiologically acceptable salts of Formula II, for example, those derived from inorganic acids such as hydrochloric, sulphuric or phosphoric acid, and organic sulphuric acids, such as p-toluenesulphonic acid or methanesulphonic acid, and organic carboxylic acids such as acetic, oxalic, succinic, tartaric, citric, malic, or maleic acid.

The present invention is also directed to the use of such compounds in the manufacture of a medicament for providing such treatment. The pharmaceutical formulation according to the invention may be administered locally or systemically. Systemic administration means any mode or route of administration that results in effective levels of active ingredient appearing in the blood or at a site remote from the site of administration of said active ingredient. A pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral, or topical administration. Indeed, all three types of formulation may be used simultaneously to achieve systemic administration of the active ingredient.

A pharmaceutical formulation according to the invention is administered in unit dose comprising from about 5 to about 1000 mg of active ingredient for a compound of general Formula I, to achieve an amount of 17-ketosteroid effective for a clinically effective therapy. Preferably, each unit dose comprises from about 5 to about 500 mg of the 17-ketosteroid. If a pharmaceutical formulation contains an anti-IL-10 antiserum, it is administered in unit dose comprising from about 0.2 mg to about 5 mg of antiserum (monoclonal or polyclonal) per day. When a pharmaceutical formulation according to the invention contains a compound of general Formula II, the formulation as administered has a unit dose comprising from about 0.01 mg to about 5,000 mg of active ingredient of general

Formula II, at a rate of 1 unit does to 10 unit doses per day, to achieve an amount of IL-10 inhibitor effective for a clinically effective therapy. Compounds of formula II can be administered in the range of 0.3 mg to about 80 mg per kilogram of body weight.

The dosages of IPA or IPA-like compounds will depend on many factors, including the mode of administration and the organism being treated. Dosages can be determined by known techniques, for example, by means of an appropriate pharmacological protocol that compares the activities of the subject compounds to the corresponding activity of a known agent.

According to one embodiment of the invention, a combination therapy is administered at a rate of from 1 unit dose to 10 unit doses each of a 17-ketosteroid compound and an IL-10 inhibitor per day. Administration of the therapy in accordance with the invention is continued for a period of at least one day and in certain cases may be given for one week, one month, or for the life of the individual. Depending on the patient's medical requirements, the therapy may be administered at intervals of once a week or every other day.

In one embodiment, an article of manufacture comprises packaging material, at least one unit dose of a 17-ketosteroid compound and a label or package insert indicating that the 17-ketosteroid compound can be used in a method as described herein. The packaging material can be made from one or more generally known materials, e.g., foam, cardboard, fibreboard, polystyrene and

polypropylene, and is of a size suitable to contain the compound(s) accompanying the packaging material. A label or package insert can be a tag or label secured to the packaging material, a label printed on the packaging material or a label inserted within the packaging material. The label indicates that the 17-ketosteroid can be used in a therapy as disclosed herein, e.g., in combination with an IL-10 inhibitor. The label can also indicate that the compound(s) have received approval from an official agency, for example, the U.S. Food and Drug Administration, for medical or veterinary use according to the method. The label may also indicate suitable administration routes, dosage regimen, and the like. If desired, the article may contain additional components such as at least one unit dose of an IL-10 inhibitor or at least one unit dose of a different 17-ketosteroid.

In another embodiment, an article of manufacture comprises packaging material, at least one unit dose of an Interleukin-10 inhibitor and a label or package insert indicating that the Interleukin-10 inhibitor can be used in a method as disclosed herein. In this embodiment, the label indicates that the IL-10 inhibitor can be used in a therapy as disclosed herein, e.g., in combination with a 17-ketosteroid. The label can also indicate that the compound(s) have received approval from an official agency, for example, the U.S. Food and Drug Administration, for medical or veterinary use according to the method. The article may contain, if desired, additional components, such as at least one unit dose of

a 17-ketosteroid or at least one unit dose of a different IL-10 inhibitor.

In another aspect of the invention, a patient suffering from an autoimmune condition is treated by administering a compound effective for increasing the amount or activity of IL-10. Such compounds are referred to herein as IL-10 effectors. Autoimmune conditions include, for example, bone marrow transplant rejection, graft versus host disease, lupus, and multiple sclerosis. It has been discovered that administration of IL-10 effectors results in a dramatic improvement in patients suffering from such autoimmune conditions. IL-10 effectors have also been discovered to enhance the antibody response in patients who suffer from a reduced immune response to a vaccine, e.g., elderly patients or very young patients. Elderly patients may have a reduced response to influenza vaccine, flu vaccine, or hepatitis B vaccine, for example.

Suitable IL-10 effectors include purified IL-10 and the polypeptide encoded by the Epstein Barr virus open reading frame (ORF) BCRF1. Purified IL-10, as well as other interleukins such as IL-12, can be prepared from a native source such as blood. However, purified interleukins such as IL-10 and IL-12 preferably are prepared from an *in vitro* cell culture expressing the desired interleukin at high levels. Interleukins can be expressed from a endogenous gene and/or from a recombinant gene introduced into the cultured cells under the control of a constitutive or inducible regulatory

element. The interleukin is purified, stored and packaged until use under conditions known in the art.

An IL-10 effector can also be a protein having conservative amino acid substitutions relative to the native IL-10 amino acid sequence, short deletions, in-frame fusions and other modifications that do not alter the biological function of IL-10.

In another aspect of the invention, a method of enhancing the T_H2 immune response in a patient comprises the step of administering an effective amount of a 17-ketosteroid compound and an effective amount of a compound that inhibits synthesis or biological activity of IL-12. Compounds that inhibit IL-12 synthesis can be determined by, for example, IL-12 specific ELISA and can include compounds that specifically alter transcription of IL-12 DNA, translation of IL-12 RNA, or processing of IL-12 protein. Compounds that inhibit the biological activity of IL-12 can include compounds that inhibit cyclic AMP activity in addition to demonstrating IL-12 inhibition in an IL-12 screening protocol. The method is useful for those patients whose response to 17-ketosteroid monotherapy is detrimentally affected by unwanted enhancement of a T_H1 immune response (i.e., production of T_H1 cytokines such as IFN, IL-2, and IL-12).

An IL-12 inhibitor can include, without limitation, antiserum to IL-12 or an IL-12 receptor molecule blocking agent. Antiserum to IL-12 can be either polyclonal or monoclonal in origin and can be an

antibody fragment having specificity and binding capacity substantially similar to intact anti-IL-12 antibody.

In another aspect of the invention, a method of enhancing the T_H2 immune response in a patient comprises the step of administering an effective amount of a 17-ketosteroid compound and an effective amount of a compound that inhibits synthesis or biological activity of interleukin-2 (IL-2). Compounds that inhibit IL-2 synthesis can be determined by, for example, IL-2 specific ELISA and can include compounds that specifically alter transcription of IL-2 DNA, translation of IL-2 RNA, or processing of IL-2 protein. Compounds that inhibit the biological activity of IL-2 can include compounds that inhibit cyclic AMP activity in addition to demonstrating IL-2 inhibition in a standard IL-2 assay. Similar to the effect observed when an IL-12 inhibitor is used, this method is useful for those patients whose response to 17-ketosteroid monotherapy is detrimentally affected by unwanted enhancement of the T_H1 immune response.

An IL-2 inhibitor can include, without limitation, antiserum to IL-2 or an IL-2 receptor molecule blocking agent. Antiserum to IL-2 can be either polyclonal or monoclonal. Antibody fragments that retain binding specificity for IL-2 are also useful as an IL-2 inhibitor.

Patients who have unwanted enhancement of the T_H1 response are, for example, patients suffering from an autoimmune condition.

In another aspect of the invention, a method of enhancing the T_H2 immune response in a patient comprises the step of administering an effective amount of a 17-ketosteroid compound and an effective amount of an T_H1 immunosuppressive peptides of human, bacterial, viral, or synthetic organ, such as alpha-fetoprotein (AFP). For example, a method of enhancing the T_H1 immune protective response in a patient comprises administration of a 17-ketosteroid compound and antiserum to human AFP. Administration of a combination therapy of a 17-ketosteroid compound and antiserum against AFP is useful, for example, when it is desired to enhance the patient's response to 17-ketosteroid monotherapy as an anti-viral, anti-bacterial, anti-mycoplasma, or anti-parasitic agent. Human alpha-fetoprotein antiserum can be either polyclonal or monoclonal in origin. Antibody fragments that retain specificity and binding capacity substantially similar to intact anti-AFP antibody are also useful.

In another aspect of the invention, a method of treating cancer, viral infection, metastasis, multi-drug resistant cancer, multi-drug resistant bacterial infection and non-drug resistant bacterial infection in a patient in need of such treatment, comprises the step of administering an interleukin-12 effector and an IL-10 inhibitor.

An IL-12 effector can be, for example, recombinant human IL-12. Human IL-12 is a disulfide-bonded heterodimeric cytokine consisting of a 40- and 35-kD

subunit. The genes for this cytokine have been cloned and purified recombinant protein has been produced. An IL-10 inhibitor useful in this aspect of the invention is similar to those indicated herein.

Another aspect of the invention includes administering a 17-ketosteroid and a lysosomotropic agent to a patient in need of treatment for a disease. A lysosomotropic agent includes, without limitation, amantadine, tributylamine, chloroquine, methylamine, quinacrine and primaquine. Another aspect of the invention includes administering a 17-ketosteroid and NG-monomethyl-l-arginine (L-NMMA) or sodium nitroprusside.

Without being bound by theory, it is believed that 17-ketosteroids, such as DHEA, have sometimes produced variable responses when administered as a monotherapy for specific conditions (e.g., lupus, multiple sclerosis, and HIV) because of patient-to-patient variation in cytokine profiles and immune reactions that occur in the patient after administration of the steroid. It is now believed that the therapeutic effectiveness of a 17-ketosteroid depends on the cytokine profile of the patient during and prior to 17-ketosteroid monotherapy. When such a steroid is first administered, the immune therapeutic response is very much patient-specific. The present invention advantageously utilizes a combination therapy, e.g., administration of a 17-ketosteroid and an IL-10 inhibitor when an enhanced T_H1 response to produce a more consistent therapeutic benefit. It is believed that such a combination therapy renders patient responses more

predictable by reducing or eliminating unwanted elevation of IL-10, a characteristic of Th_2 immune responses. Such a combination therapy facilitates enhancement of IL-12 mediated immune responses without the general negative effects of concomitantly enhancing IL-10 mediated immune responses.

On the other hand, it is sometimes desirable to enhance the T_H2 immune response, e.g., in an autoimmune condition. In such situations, a combination therapy comprising a 17-ketosteroid compound and an IL-10 effector can be advantageously utilized. It is believed that such a combination therapy produces a more consistent therapeutic benefit by enhancing IL-10 mediated immune responses without the general negative effects of concomitantly enhancing IL-12 mediated immune responses.

The invention will be further understood with reference to the following illustrative embodiments, which are purely exemplary and should not be taken as limiting the true scope of the present invention as described in the claims. All publications, patents and other references mentioned herein are incorporated by reference in their entirety.

EXAMPLES

Example 1

Effect of Administering DHEA Monotherapy to HIV Patients

Experimental evidence using DHEA therapy in HIV+ patients has demonstrated that IL-12 levels, as measured

by antibody ELISA methods, are elevated; natural killer cell levels are increased together with the synthesis and presence of gamma- interferon (γ-IFN); and HIV viral loads, as measured by HIV PCR (RNA) measurement and quantitative culturing techniques, more than one log after four weeks of DHEA monotherapy. While viral load levels were considerably reduced, however, T_H1 immune response improvement did not occur. In fact, due to the elevated levels of IL-12 generated by this monotherapy with DHEA, IL-10 levels increased causing a subsequent decline in CD4+ cell numbers and the disappearance of a T_H1 immune response (Delayed Type Hypersensitivity Response). Skin reaction in patients as evidenced by patient data was down-regulated by DHEA monotherapy, contrary to previous beliefs of some. Skin reaction is only restored by the removal of IL-10 which is elevated by the DHEA monotherapy.

The following is a summary of using DHEA as a monotherapy in an open-label dose-escalation trial of oral DHEA tolerance and pharmacokinetics in patients with HIV disease. In the Phase I DHEA trial (early symptomatic HIV disease and 200 to 500 CD4+ lymphocytes/μL), absolute CD4 counts in the control, placebo-assigned patients declined by a median 5 cell/month. In contrast, patients in the lowest-dose group studied in the Phase I DHEA trial (whose immune system would not be expected to decline faster than that of placebo-treated patients in the other trial) had a median CD4+ decline of 31 cells/month. Substantial

therapeutic benefit could not be accomplished by the use of DHEA alone.

Example 2

Effect of Administering DHEA in Combination with an IL-10 Inhibitor to HIV Patients

In-Vivo Trial using Combination Therapy

To counteract the T_H1 suppressive immune side effect of DHEA monotherapy, the steroid was combined with an agent to inhibit or interrupt the synthesis and/or action of IL-10. This combination therapy is the preferred embodiment of using compounds according to general formula I when it is desired for the 17-ketosteroid to generate a Th_1 immune response. The component used to counteract the Th_2 suppressive IL-10 immune side effect was rabbit polyclonal antiserum against human IL-10.

When the combination therapy was administered to HIV+ patients, the removal of viral particles from each patient's bloodstream was enhanced by three logs relative to DHEA monotherapy. At the same time, the CD4+ helper T-cell count increased by over 80% with combination therapy. The Delayed Type Hypersensitivity response lost at sero-conversion was also restored with combination therapy. The beneficial action of administering a 17-ketosteroid and an IL-10 inhibitor has wider therapeutic usefulness than solely for treatment of HIV.

Example 3

Administration of DHEA and IL-10 Inhibitor to Patients Having T_H1 Autoimmune Conditions

The therapeutic benefit of DHEA therapy to lupus patients and to other T_H1 autoimmune conditions is directly related to the increase of endogenous IL-10 levels achieved in the patient by the administration of DHEA. Bone marrow transplant rejection was put into remission by DHEA administration to enhance IL-10 levels.

A patient (RD, date of birth: 14/7/1983) had Acute Myeloid Leukaemia M3 in remission following allogenic bone marrow transplant. His major active problems were GUT Graft Versus Host Disease (GVHD) and severe lung disease. A physician's report indicated that RD's general health had improved over a three month period that coincided with administration of therapy for 14 days and he is now enjoying good health. For the first time since his diagnosis he has been able to enjoy full days at school. He no longer needs nasal gastric feeds or suffers with diarrhea. His lung function remains at 30% but his exercise tolerance has improved dramatically. He no longer needs a wheelchair and can tolerate light exercise. As he is on no other drug regime and has been taking this medication for nearly three months, we must consider that this therapy is influencing these beneficial effects on his body. Before commencing the therapy, he was nebulizing Ventolin, Atrovent, and Pulmicort four times per day, with oral steroids when necessary. Now he nebulizes only twice a day. I have

never seen such a vast improvement in his health with no apparent side effects.

Example 4

Administration of IL-10 to Patients Having an Autoimmune Condition

In clinical experiments, patients who had elevated endogenous IL-10 levels also experienced remission in lupus, whereas patients who, due to other cytokine and immune factors, have not experienced an IL-10 elevation have not demonstrated relief of symptoms. Therefore, a means of achieving relief of symptoms for such autoimmune conditions is to administer IL-10 in combination with a 17-ketosteroid to facilitate the remission of symptoms of autoimmune diseases such as lupus and GVHD. Experiments with DHEA and measurement of the cytokine profiles of patients who respond to DHEA therapy and those who do not respond to DHEA therapy have led to the discovery that elevated IL-10 is the active agent responsible for the alleviation of the clinical symptoms of lupus.

Multiple Sclerosis (MS) is believed to be an autoimmune condition. Treatment of MS patients with DHEA monotherapy results in high patient-to-patient variability, similar to the variability observed with lupus. Remission of symptoms in MS was identified in those patients who experienced significant elevation of their endogenous levels of IL-10. Further, direct admission of recombinant IL-10 to a MS model in the Lewis rat demonstrated remission of symptoms. If IL-10 is

administered prior to the onset of myelin damage the symptoms of multiple sclerosis are prevented altogether.

Example 5

Administration of IL-10 to Patients Having Reduced Vaccine Response

Another area of therapeutic benefit ascribed to DHEA is the enhancement of vaccine antigen recognition by the immune system in the elderly. Analysis of IL-10 levels in elderly patients receiving DHEA in conjunction with a vaccine indicated that those patients having elevated IL-10 levels had enhanced antigen recognition compared to patients not having elevated IL-10 levels. This result suggested that use of IL-10 as a vaccine adjuvant would be useful. Normally, elderly patients would be expected to have a reduced antigen "vaccine take" or immune response due to age.

Administration of recombinant IL-10 to elderly patients in association with or in advance of treatment with an antigen vaccine created an enhanced adjuvant effect and directly enhanced the antibody response. Administration of recombinant IL-10 in conjunction with vaccine administration was a means of achieving enhanced antibody response in patients having a reduced vaccine response (e.g., elderly or very young patients). Administration of IL-10 or an IL-10 effector removes the uncertainties associated with the use of DHEA.

Experimental autoimmune encephalomyelitis (EAE) can be induced in a variety of rodent strains and is

widely used as a model for MS. Recombinant IL-10 was administered to EAE-induced Lewis rats. After administering IL-10, animals were observed for symptoms of MS. All animals receiving IL-10 showed complete remission of symptoms. All animals receiving mock injections showed no change in symptoms or a deterioration in symptoms.

Example 6

Administration of IL-12 to HIV Patients

In vitro DHEA IL-12 Study with HIV + Blood

Protocol to demonstrate that DHEA enhances endogenous levels of IL-12

Restoration of HIV-Specific Cell-Mediated Immune Responses by DHEA

Blood samples from four patients were tested for the effect of DHEA or IL-12 in the presence of HIV gp120. One HIV-1 negative control (E9B) and three HIV-1 positive specimens (E9C, E9E and E9F) were stimulated by the addition of DHEA or IL-12 in the presence of gp120. Data for these samples is shown in Tables 1-18.

Table 1

ASSAY PARAMETERS

Test Reagent: Dehydroisoandrosterone (DHEA) Sigma D-4000

Test Cells: Mononuclear cells from five HIV-1 positive donors

Mononuclear cells from two HIV-1 negative donors

200 µL culture; at least 3 replicates of each experimental condition

Basic Medium; RPMI 1640. 10% FBS, 50 µL gentamicin

Sample	HIV-1 status	Total Cells	Number of Plates	Cells/mL	Cells/culture	CD4 count
E9A	negative	1 x 10^7	2	2.5 x 10^5	5 x 10^4	not provided
E9B	negative	1 x 10^7	2	2.5 x 10^5	5 x 10^4	not provided
E9C	positive	7.1 x 10^6	1	3.55 x 10^5	7.1 x 10^4	not provided
E9D	positive	1.02 x 10^7	1	5.1 x 10^5	1.02 x 10^5	not provided
E9E	positive	9.65 x 10^6	1	4.83 x 10^5	9.7 x 10^4	not provided
E9F	positive	1.08 x 10^7	1	8.6 x 10^5	1.73 x 10^5	526
E9G	positive	8.45 x 10^6	1	7.04 x 10^5	1.41 x 10^5	758
Samples E9F and E9G were plated with fewer cultures to increase the number of cells per culture						

Table 2A
Sample E9C

Values reported are optical density (O.D.)

7 days; MTS Cell Proliferation Assay 2h incubation

	----------with gp120----------									% of gp120 Control	
	1	2	3	4	5	6	7	8	Avg.	gp120	no gp120
DHEA 10^{-4}M	0.177	0.253	0.319	0.479	0.521	0.750	0.486	0.262	0.407	72.8	80.7
DHEA 10^{-6}M	0.227	0.433	0.538	0.685	0.595	0.679	0.697	0.845	0.588	105.1	76.0
DHEA 10^{-8}M	0.325	0.601	0.608	0.570	0.724	0.739	1.053	0.819	0.680	121.6	112.5
DHEA 10^-8M + anti IL-12	0.302	0.425	0.542	0.554	0.543	0.489	0.787	0.804	0.558	99.4	137.3
DHEA 10^-10M	0.336	0.513	0.530	0.572	0.505	0.554	0.726	0.608	0.543	97.1	70.6
anti IL-12	0.281	0.412	0.513	0.718					0.481	86.0	116.5
IL-12	0.282	0.494	0.911	0.716	0.644	0.624	0.839	0.735	0.681	121.8	144.6
No Reagent	0.270	0.474	0.395	0.612	0.654	0.576	0.684	0.809	0.559	100.0	67.3

Table 2B

	----------without gp 120----------					% of
	1	2	3	4	Average	gp 120 Control
DHEA 10^-4M	0.604	0.238	0.245	0.717	0.451	80.7
DHEA 10^-6M	0.423	0.426	0.465	0.386	0.425	76.0
DHEA 10^-8M	0.400	0.617	0.523	0.975	0.629	112.5
DHEA 10^-8M + anti IL-12	0.745	0.504	0.717	1.104	0.768	137.3
DHEA 10^-10M	0.375	0.422	0.368	0.413	0.395	70.6
anti IL-12	0.472	0.619	0.582	0.931	0.851	116.5
IL-12	0.843	0.700	0.701	0.989	0.808	144.6
No Reagent	0.371	0.386	0.414	0.334	0.376	67.3
PHA 1%	0.306	0.281	0.354	0.300	0.310	55.5

Table 3A
Sample E9D

7 days; MTS Cell Proliferation Assay 2.75h incubation

	----------with gp120----------									% of gp120 Control	
	1	2	3	4	5	6	7	8	Avg.	gp120	no gp120
DHEA 10^-4M	0.689	0.320	0.720	0.804	0.690	0.443	0.709	0.579	0.619	94.5	70.3
DHEA 10^-6M	0.705	0.542	0.512	0.498	0.478	0.472	0.499	0.704	0.551	84.4	48.0
DHEA 10^-8M	0.492	0.359	0.041	0.470	0.484	0.411	0.487	0.535	0.409	62.6	46.0
DHEA 10^{-8}M + anti IL-12	0.661	0.419	0.619	0.351	0.484	0.473	0.463	0.472	0.493	75.5	100.3
DHEA 10^-10M	0.688	0.476	0.432	0.398	0.391	0.431	0.414	0.419	0.456	69.9	46.0
anti IL-12					0.517	0.486	0.500	0.534	0.509	78.0	104.0
IL-12	0.588	0.548	0.378	0.447	0.565	0.451	0.525	0.608	0.514	78.7	47.5
No Reagent	0.825	0.704	0.561	0.605	0.717	0.605	0.561	0.649	0.653	100.0	44.4

Table 3B

	----------without gp 120----------					% of
	1	2	3	4	Average	gp 120 Control
DHEA 10^-4M	0.520	0.292	0.522	0.501	0.459	70.3
DHEA 10^-6M	0.291	0.286	0.367	0.310	0.314	48.0
DHEA 10^-8M	0.300	0.362	0.244	0.295	0.300	46.0
DHEA 10^-8M + anti IL-12	0.491	0.597	0.642	0.880	0.655	100.3
DHEA 10^-10M	0.296	0.256	0.416	0.233	0.300	46.0
anti IL-12	0.517	0.662	0.759	0.778	0.679	104.0
IL-12	0.264	0.259	0.347	0.370	0.310	47.5
No Reagent	0.328	0.314	0.276	0.241	0.290	44.4
PHA 1%	0.733	0.642	0.582	0.495	0.613	93.9

Table 4A
Sample E9E

Report of Cellular Proliferation MTS Assay

7 days; MTS Cell Proliferation Assay 2.75h incubation

	----------with gp120----------									% of gp120 Control	
	1	2	3	4	5	6	7	8	Avg.	gp120	no gp120
DHEA 10^-4M	0.653	0.926	0.948	0.645	0.778	0.758	0.828	0.860	0.799	122.4	73.2
DHEA 10^-6M	0.919	0.870	0.939	1.131	1.068	0.725	0.739	0.943	0.916	140.2	39.8
DHEA 10^-8M	1.001	0.820	0.860	0.634	0.827	0.703	0.739	0.890	0.784	120.1	37.9
DHEA 10^-8M + anti IL-12	0.909	0.880	1.368	0.864	0.873	1.238	1.375	0.648	1.017	155.7	84.7
DHEA 10^-10M	0.988	0.764	0.638	0.715	0.718	1.069	0.781	0.618	0.788	120.4	46.7
anti IL-12					0.783	1.091	0.873	0.753	0.875	134.0	97.7
IL-12	1.195	1.102	0.822	0.781	0.918	0.891	0.854	0.812	0.922	141.2	80.7
No Reagent	1.114	1.051	0.821	0.924	1.004	0.940	0.783	0.735	0.923	100.0	64.8

Table 4B

	----------without gp 120----------					% of
	1	2	3	4	Average	gp 120 Control
DHEA 10^-4M	0.562	0.608	0.735	0.800	0.675	73.2
DHEA 10^-6M	0.413	0.336	0.400	0.320	0.387	39.8
DHEA 10^-8M	0.361	0.385	0.321	0.332	0.350	37.9
DHEA 10^-8M +anti IL-12	0.721	0.783	0.713	0.910	0.782	84.7
DHEA 10^-10M	0.551	0.299	0.473	0.402	0.431	46.7
anti IL-12	0.787	0.905	0.867	1.047	0.902	97.7
IL-12	0.416	0.991	1.102	0.469	0.745	80.7
No Reagent	0.607	0.480	0.579	0.726	0.588	84.3
PHA 1%	0.37	0.487	0.355	0.45	0.418	45.3

Table 5A

Sample E9F

7 days; MTS Cell Proliferation Assay 4h incubation
CD4 41%

	with gp120							% of gp120 Control	
	1	2	3	4	5	6	Avg.	gp120	no gp120
DHEA 10^-4M	1.227	1.309	1.302	1.288	1.130	1.393	1.275	116.3	98.1
DHEA 10^-8M	1.120	1.062	1.038	1.082	1.163	1.167	1.105	100.9	69.7
DHEA 10^-8M + aIL-12	1.133	1.356	1.145	1.204	1.157	1.232	1.208	110.0	86.2
antiIL-12				0.958	1.053	1.106	1.042	95.1	87.1
IL-12	1.085	1.071	1.038	1.179	1.052	1.024	1.075	98.1	63.5
No Reagent	1.035	1.075	1.004	1.195	1.136	1.132	1.096	100.0	56.3

Table 5B

	----------without gp120----------					% of
	1	2	3	4	Average	gp120 Control
DHEA 10^-4M	1.122	0.865	1.201	0.984	1.046	98.1
DHEA 10^-8M	0.659	0.628	1.036	0.647	0.743	69.7
DHEA 10^-8M + anti IL-12	1.014	0.914	0.907	0.927	0.941	85.2
anti IL-12	0.924	0.965	0.897	0.929	0.929	87.1
IL-12	0.638	0.693	0.833	0.549	0.675	63.6
No Reagent	0.510	0.591	0.69	0.589	0.590	55.3
PHA 1%	0.646	0.716	0.595		0.653	70.7

Table 6A
Sample E9G

7 days; MTS Cell Proliferation Assay 4h incubation
CD4 41%

	----------with gp120----------							% of gp120 Control	
	1	2	3	4	5	6	Avg.	gp120	no gp120
DHEA 10^-4M	1.243	1.270	1.229	1.351	1.230	1.268	1.265	97.2	104.1
DHEA 10^-8M	1.037	1.316	1.234	1.156	1.371	1.394	1.236	95.0	57.6
DHEA 10^-8M+ anti IL-12	1.325	0.692	1.092	1.172	1.271	1.332	1.147	86.1	90.5
anti IL-12				0.989	1.333	1.532	1.268	98.9	86.4
IL-12	1.404	1.204	1.273	1.225	1.248	1.320	1.279	98.2	29.3
No Reagent	1.373	1.279	1.229	1.361	1.325	1.243	1.302	100.0	46.6

Table 6B

	----------without gp120----------					% of
	1	2	3	4	Average	gp 120 Control
DHEA 10^-4M	1.416	1.290	1.293	1.421	1.355	104.1
DHEA 10^-8M	0.644	0.700	0.967	0.690	0.750	57.6
DHEA 10^-8M +anti IL-12	1.046	1.194	1.410	1.063	1.178	90.6
anti IL-12	1.031	1.262	1.197	1.009	1.125	86.4
IL-12	0.357	0.421	0.402	0.347	0.382	29.5
No Reagent	0.415	0.424	0.416	1.171	0.607	46.6
PHA 1%	0.478	0.559	0.571		0.638	41.2

Table 7A
Sample E9A

5 days; MTS Cell Proliferation Assay 4h incubation
No anti IL-2 receptor in plate

	----------with gp120----------									% of gp120 Control	
	1	2	3	4	5	6	7	8	Avg.	gp120	no gp 120
DHEA 10^-4M	1.125	0.861	0.957	0.974	0.969	0.917	0.918	0.887	0.954	95.5	51.4
DHEA 10^-6M	0.861	0.778	0.841	0.791	0.826	0.785	0.872	0.893	0.831	83.3	51.5
DHEA 10^-8M	0.871	0.803	0.768	0.812	0.820	0.807	0.886	0.749	0.815	81.6	45.8
DHEA 10^-8M+ aIL-12	1.060	0.795	0.785	0.775	0.726	0.758	0.750	0.843	0.812	81.3	62.8
DHEA 10^-10M	1.008	0.820	0.782	0.747	0.805	0.746	0.850	0.747	0.788	79.0	39.6
DHEA 10^-12M	0.944	0.740	0.682	0.740	0.891	0.731	0.734	0.868	0.766	76.8	40.2
IL-12	0.943	0.877	0.842	0.872	0.798	0.848	0.873	0.797	0.856	85.8	58.5
No Reagent	0.894	1.097	0.901	1.040	1.028	0.962	0.977	1.083	0.988	100	42.8

Table 7B

	----------without gp 120----------					% of
	1	2	3	4	Average	gp120 Control
DHEA 10^-4M	0.487	0.488	0.515	0.582	0.513	51.4
DHEA 10^6M	0.643	0.575	0.107	0.514	0.535	53.8
DHEA 10^-8M	0.458	0.505	0.434	0.432	0.457	45.8
DHEA 10^-8M +anti IL-12	0.468	0.713	0.578	0.747	0.627	62.8
DHEA 10^-10M	0.387	0.376	0.388	0.431	0.395	39.6
DHEA 10^-12M	0.399	0.362	0.391	0.454	0.402	40.3
IL-12	0.666	0.576	0.660	0.434	0.584	58.5
No Reagent	0.423	0.423	0.402	0.481	0.427	42.8

Table 8A
Sample E9A

7 days; MTS Cell Proliferation Assay 4h incubation
With anti IL-2 receptor in plate

	---------- OD WITH gp 120----------									% of gp 120 Control	
	1	2	3	4	5	6	7	8	Avg.	gp 120	ng gp 120
DHEA 10^-4M	1.123	0.601	0.739	0.555	0.653	0.873	0.719	0.880	0.743	80.2	87.8
DHEA 10^-6M	0.969	0.449	0.763	0.791	0.767	0.800	0.378	0.536	0.882	73.6	58.1
DHEA 10^-8M	0.704	0.497	0.461	0.401	0.708	0.501	0.865	0.809	0.693	84.1	73.4
DHEA 10^-8M+ all.-12	0.769	0.307	0.823	0.543	0.567	0.419	0.703	0.924	0.634	68.5	91.7
DHEA 10^-10M	0.851	0.419	0.644	0.421	0.541	0.533	0.689	0.655	0.598	64.8	57.7
DHEA 10^-12M	0.799	0.548	0.420	0.547	0.485	0.439	0.598	0.628	0.558	60.3	58.5
IL-12	1.041	0.879	0.826	0.518	0.740	0.579	0.780	0.783	0.743	80.3	74.6
No Reagent Control	0.758	1.037	0.890	0.633	0.994	0.974	0.896	1.222	0.926	100	65.9

Table 8B

	----------OD WITHOUT gp120----------					% of
	1	2	3	4	Average	gp120 Control
DHEA 10^-4M	0.905	0.711	0.956	0.681	0.813	87.6
DHEA 10^6M	0.583	0.431	0.555	0.602	0.538	58.1
DHEA 10^-8M	0.696	0.955	0.574	0.495	0.680	73.4
DHEA 10^-8M +anti IL-12	0.691	0.823	0.747	0.935	0.849	91.7
DHEA 10^-10M	0.595	0.486	0.476	0.580	0.534	57.7
DHEA 10^-12M	0.562	0.497	0.631	0.475	0.541	58.5
IL-12	0.776	0.692	0.617	0.880	0.691	74.6
No Reagent	0.684	0.603	0.565	0.590	0.611	85.9

Table 9A
Sample E9B

3 days, MTS Cell Proliferation assay 3h incubation

	OD WITH gp 120							% of gp 120 control	
	1	2	3	4	5	6	Average	gp120	no gp 120
DHEA 10^-4 M	0.663	0.668	0.568	0.661	0.634	0.626	0.637	139.9	120.8
DHEA 10^-6 M	0.577	0.593	0.708	0.667	0.728	0.676	0.658	144.7	111.3
DHEA 10^-8 M	0.650	0.621	0.708	0.685	0.692	0.703	0.677	148.7	110.9
DHEA 10^-8 M + alL-12	0.549	0.719	0.712	0.674	0.717	0.742	0.702	154.3	173.4
DHEA 10^-10 M	0.707	0.683	0.691	0.670	0.654	0.648	0.676	148.5	122.3
DHEA 10^-12 M	0.655	0.619	0.632	0.654	0.638	0.660	0.643	141.3	110.5
IL-12	0.672	0.652	0.665	0.618	0.669	0.689	0.661	145.2	101.3
No Reagent	0.640	0.415	0.398	0.431	0.427	0.419	0.455	100.0	113.6
anti IL-2 Receptor?	YES	YES	YES	NO	NO	NO			

Table 9B

	OD WITHOUT gp120					% of gp 120 Control
	1	2	3	4	Average	
DHEA 10^{A} -4M	0.581		0.595	0.49	0.532	0.550	120.8
DHEA 10^{A} -6M	0.494		0.517	0.536	0.478	0.506	111.3
DHEA 10^{A} -8M	0.560		0.493	0.475	0.491	0.505	110.9
DHEA 10^{A} -8M + anti IL-12	0.823		0.775	0.775	0.783	0.789	173.4
DHEA 10^{A} -10M	0.556		0.562	0.559	0.548	0.556	122.3
DHEA 10^{A} -12M	0.531		0.496	0.466	0.528	0.503	110.5
IL-12	0.328		0.485	0.542	0.489	0.461	101.3
No Reagent	0.547		0.485	0.523	0.513	0.517	113.6
anti IL-2 Receptor?	YES		YES	NO	NO		
PHA 1% +anti IL-2 Receptor	0.610		0.862	0.654	0.625	0.638	140.2
PHA 1%	0.601		0.672	0.644	0.689	0.662	143.2

Table 10A
Sample E9B

6 days, MTS Cell Proliferation assay 3h incubation

	OD WITH gp 120							% of gp 120 control	
	1	2	3	4	5	6	Average	gp120	no gp 120
DHEA 10^ -4 M	0.903	0.963	1.318	1.398		0.817	1.080	124.0	68.9
DHEA 10^ -6 M	0.601	0.805	0.797	0.963	0.740	1.004	0.817	93.8	77.5
DHEA 10^ -8 M	0.847	0.748	0.770	0.687	0.815		0.773	88.8	55.4
DHEA 10^ -8 M + anti IL-12	0.871	0.976	0.897	0.972	0.956	1.332	1.001	114.9	104.9
DHEA 10^ -10 M	0.938	0.727	0.820	0.823	0.850	0.657	0.803	92.1	60.4
DHEA 10^ -12 M	0.959	0.618	0.678	0.771	0.687	0.865	0.763	87.6	59.6
IL-12	0.769	0.898	0.911	0.864	0.894	0.755	0.852	97.8	58.9
No Reagent Control	1.012	0.907	0.694	0.871	0.963	0.777	0.871	100.0	61.3
anti IL-2 Receptor?	YES	YES	YES	NO	NO	NO			

Table 10B

	OD WITHOUT gp120					% of gp 120 Control
	1	2	3	4	Average	
DHEA 10^ -4M	0.538	0.520	0.605	0.737	0.800	68.9
DHEA 10^ -6M	0.962	0.570	0.481	0.687	0.675	77.5
DHEA 10^ -8M	0.566	0.524	0.375	0.454	0.482	55.4
DHEA 10^ -8M + anti IL-12	0.996	0.913	1.045	0.700	0.914	104.9
DHEA 10^ -10M	0.566	0.578	0.420	0.541	0.526	60.4
DHEA 10^ -12M	0.584	0.546	0.438	0.510	0.520	59.6
IL-12	0.580	0.612	0.423	0.436	0.513	58.9
No Reagent	0.761	0.350	0.419	0.605	0.534	61.3
anti IL-2 Receptor?	YES	YES	NO	NO		
PHA 1% + anti IL-2 Receptor	0.609	0.553	0.548	0.586	0.574	65.9
PHA 1%	0.567	0.635	0.546	0.524	0.568	65.2

Table 11A
DATA SUMMARY FOR SAMPLES E9B, E9A, E9C AND E9D

	HIV-1- Blood E9B % of gp120 control		HIV-1- Blood E9A % of gp120 control		HIV-1+ Blood E9C % of gp120 control		HIV-1+ Blood E9D % of gp120 control	
	gp120	no gp120	gp120	no gp120	gp120	no gp120	gp120	no gp120
DHEA 10^-4M	124.0	88.9	80.2	87.8	72.8	80.7	94.8	70.3
DHEA 10^-6M	93.8	77.5	73.6	58.1	105.1	76.0	84.4	48.0
DHEA 10^-8M	88.8	55.4	61.1	73.4	121.6	112.5	62.6	46.0
DHEA 10^-8M + anti IL-12	114.9	104.9	68.5	91.7	99.4	137.3	75.5	100.3
DHEA 10^-10M	92.1	60.4	64.6	57.7	97.1	70.6	69.9	46.0
DHEA 10^-12M	87.6	59.6	60.3	58.5				
anti IL-12					86.0	116.5	78.0	104.0
IL-12	97.8	58.9	80.3	74.6	121.8	144.6	78.7	47.5
NoReagent	100.0	61.3	100	66.9	100.0	67.3	100.0	44.4
PHA 1%		65.2				55.5		93.9

Table 11B
DATA SUMMARY FOR SAMPLES E9E, E9F AND E9G

	E9E % of gp120 control		E9F % of gp120 control		E9G % of gp120 control	
	gp120	no gp120	gp120	no gp120	gp120	no gp120
DHEA 10^ -4M	122.4	73.2	116.3	98.1	97.2	104.1
DHEA 10^ -6M	140.2	89.8				
DHEA 10^ -8M	120.1	37.9	100.9	69.7	95.0	57.6
DHEA 10^ -8M + anti IL-12	155.7	84.7	110.0	88.2	88.1	90.5
DHEA 10^ -10M	120.4	46.7				
anti IL-12	134.0	97.7	95.1	87.1	98.9	86.4
IL-12	141.2	80.7	98.1	63.6	98.2	29.3
NoReagent	100.0	64.8	100.0	55.3	100.0	46.6
PHA 1%		45.3		70.7		41.2

Table 12
IL-2 ELISA for E9C HIV+

Blood E9C HIV-1+	pg/mL with gp 120			pg/mL without gp 120	
	1	2	3	1	2
DHEA 10^ -4M	0	0	0	0	0.4
DHEA 10^ -6M	0.4	0	0	0	0
DHEA 10^ -8M	0	0	0.9	0	0
DHEA 10^ -8M + anit IL-12	0	0	0	0	0
DHEA 10^ -10M	0	0	0	0	0
anti-IL-12	0	0	0	0	0
IL-12	0.9	0	0	0	0
No Reagent	0	0	0	0	0
PHA 1% 7 days				0	0
PHA 1% 3 days				14.7	10.9

Table 13
IL2-ELISA for E9D HIV+

Blood E9D HIV-1+	pg/mL with gp 120			pg/mL without gp 120	
	1	2	3	1	2
DHEA 10^{A} -4M	0	0	0	0	0
DHEA 10^{A} -6M	0	0	0	0	0
DHEA 10^{A} -8M	0	0	2.5	0	0
DHEA 10^{A} -8M + alL-12	0	0	0	0	0
DHEA 10^{A} -10M	0	0	0	0	0
anti-IL-12	0	0	0	0	0
IL-12	0	0	0.1	0	0
No Reagent	0	0	0	0	0
PHA 1% 7 days				0	0
PHA 1% 3 days				34.2	35.1

Table 14
IL-2 ELISA for E9E HIV+

	pg/mL with gp 120			pg/mL without gp 120	
	1	2	3	1	2
DHEA 10^{A} -4M	0	0	0	0	0
DHEA 10^{A} -6M	0	0	1.7	3.3	0
DHEA 10^{A} -8M	0	0	0	0	0
DHEA 10^{A} -8M + alL-12	0	0	0	0	515
DHEA 10^{A} -10M	0	0	0	0	0
anti-IL-12	0	0	0	0	0
IL-12	0	0	0	0	0
No Reagent	0	0	0	0	0
PHA 1% 7 days				0	0
PHA 1% 3 days				18.4	200.1

Table 15
IL-2 ELISA for E9F HIV+

	----pg/mL with gp 120------			pg/mL without gp 120	
	1	2	3	1	2
DHEA 10^-4 M	0	0.9	1.5	0	0.4
DHEA 10^-8M	0	0	0.9	0	0
DHEA 10^-8 M + aIL-12	0	0	0	0	0
anti-IL-12	0	0	0	0	0
IL-12	0	0	0	0	0
No Reagent Control	0	0	0	0	5.3
PHA 1% 7 days				1.8	0
PHA 1% 1 day				231.8	195.7

Table 16
IL-2 ELISA for E9F HIV+

	----pg/mL with gp 120------			pg/mL without gp 120	
	1	2	3	1	2
DHEA 10^-4 M	0.4	0.9	0	0	0.4
DHEA 10^-8M	0	0	0.9	0	0
DHEA 10^-8 M + aIL-12	2	0	0	0	0
anti-IL-12	0	2.4	0.9	0.6	0
IL-12	0	3.6	2.4	0.6	1.8
No Reagent Control	4.1	5.4	4.8	4.1	0
PHA 1% 7 days				0	0
PHA 1% 1 day				211.7	326.1

Table 17
IL-2 ELISA for E9A HIV-

	pg/mL with gp120				pg/mL	
E9A blood HIV1-	1	2	3	4	1	2
DHEA 10^-4M	0	0	0	0	0.8	0
DHEA 10^-6M	0	0	0	0	0	0
DHEA 10^-8m	0	0	0	0	0	0
DHEA 10^-8M + anti IL-12	0	0	0	0	0	0
DHEA 10^-10M	0	0	0	0	0	0
DHEA 10^-12M	0	0	0	0	0	0
IL-12	0	0	0	0	0	0
No Reagent Control	0	0	0	0	0	0

Table 18
IL-2 ELISA for E9B HIV-

Blood E9B	pg/mL with gp 120				pg/mL without gp 120			
HIV-1negative	1	2	3	4	1	2	3	4
DHEA 10^ -4M	0	0			0			
DHEA 10^ -6M	0	0			0			
DHEA 10^ -8M	0	0			0			
DHEA 10^ -8M + alL-12	0	0	0	0	0			
DHEA10^ -10M	0							
DHEA 10^ -12M	0	0			0			
IL-12	0	0			0			
No Reagent Control	0	0			0			
PHA 1% 6 days					0	0	0	0
PHA 1% 3 days					70.1	53.4	25.4	13

The stimulation caused by DHEA in each of these cases was equal to or greater than that caused by IL-12, although the concentration of DHEA causing the stimulation varied from sample to sample. In blood samples from three other patients (E9A, HIV-1 negative; E9D and E9G, HIV-1 positive), proliferation in the presence of gp120 was suppressed by addition of DHEA or IL-12.

It has recently been demonstrated that in vivo administration of murine IL-12 (IL-12) to mice results in augmentation of cytotoxic natural killer (NK)/lymphocytes-activated killer cell activity, enhancement of cytolytic T cell generation, and induction of γ-IFN secretion. In this study, the *in vivo* activity of murine IL-12 against a number of murine tumors has been evaluated. Experimental pulmonary metastases or subcutaneous growth of the B16F10 melanoma were markedly reduced in mice treated intra peritoneally with IL-12, resulting in an increase in survival time. The therapeutic effectiveness of IL-12 was dose dependent and treatment of subcutaneous tumors were effectively treated by IL-12 at doses which resulted in no gross toxicity. Local peritumoral injection of IL-12 into established subcutaneous Renca tumors resulted in regression and complete disappearance of these tumors. IL-12 was as effective in NK cell-deficient beige mice or in mice depleted of NK cell activity by treatment with anti-asialo GM1, suggesting that NK cells are not the primary cell type mediating the anti-tumor effects of this

cytokine. However, the efficacy of IL-12 was greatly reduced in nude mice, suggesting the involvement of T cells. Furthermore, depletion of CD8+ but not CD4+ T cells significantly reduced the efficacy of IL-12. These results demonstrate that IL-12 has potent *in vivo* anti-tumor and anti-metastatic effects against murine tumors and demonstrate the critical role of CD8+ T- cells in mediating the anti-tumor effects against subcutaneous tumors.

The involvement of IL-12 with the generation of CD8+ cell populations was demonstrated in a study of HIV+ patients who were treated with anti-human IL-10 antiserum. HIV viral load was reduced to zero by the administration of rabbit anti-human IL-10 antiserum. Patients showed an 84% increase in CD8+ cell population above baseline values. The decrease in *in vivo* IL-10 levels allowed CD8+ cell populations to increase and allowed for HIV viral clearance by restoring HIV specific cell mediated immune response.

PRODUCT SPECIFICATIONS

Description:	Rabbit anti-Human IL-10
Form:	Liquid
Concentration:	2.7 mg/ml
Stabilizers:	None
Preservative:	None
Sterility	Sterile filtered
Host Species:	Rabbit
Antibody Class:	IgG

Antigen Used:	Recombinant human IL-10
Method of Purification:	Ion Exchange chromatography
Method of Quantification:	Pierce BCA Protein Assay
Specificity:	Human IL-10
Cross-Reactivity:	No cross reactivity with WHO standards: IL-1a, IL-1B, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, MIP-1A, TNFa and GM-CSF done by EIA.
Storage:	Short term, 4°C; -20°C, long term.

Materials and Reagents: used to demonstrate DHEA's ability to enhance IL-12 synthesis.

1. IL-2 ELISA, available in house, minimum of six plates.

2. MTS assay, Promega, minimum of 7 plates.

3. IL-12 (R&D Systems, Minneapolis, Minnesota, #219-IL) 5 fg should be sufficient for the entire experiment.

4. Antibody to human IL-2 receptor, (R&D Systems, AB-233-NA), 1 mg lyophilized, goat human.

5. Rabbit polyclonal antibody to p40 chain of human IL-2 (Genetics Institute, Cambridge, Massachusetts).

6. Native gp120, available in house (50 fg/vial, about 1 mg/mL). Need 5nM/mL. 50 fg is enough for two assays with two plates each.

7. Normal human (HIV-1 negative). Peripheral blood mononuclear cells (PBMC) unstimulated.

8. 5 HIV+ samples of blood from which to obtain non-responsive PBMC. 5 mL per sample.

9. DHEA (dehydroisoandrosterone; Sigma Chemical, St. Louis, Missouri, D4000). 1 g should be sufficient for the entire experiment.

10. 100% Ethanol to solubilize the DHEA.

11. R10 Medium: RPMI, 10% FBS, 50 fg/mL gentamicin.

12. 96 well flat bottom tissue culture grade cluster dishes, 2 per blood sample.

Protocol:

1. For each blood sample, separate out PBMCs and do a cell count.

2. Use all the cells available from patient samples. If 1 x 10^7 cells or more are present, then seed the cells into two 96 well plates. At 1 x 10^7, we will end up with 0.5 x 10^5 cells/well or 2.5 x 10^5 cells/mL. If fewer, then use only one plate. Record the number actually plated per well. If two plates are used, then one will be for IL-12 detection and will receive antibody to human IL-2 receptor. The other plate will be used for the cell proliferation assay and will not receive this antibody. If only one plate is used, then that plate will receive antibody.

3. If using one plate, then resuspend the cells in 20 mL R10; if two plates, then resuspend in 40 mL. Aliquot

200 fL per well. Allow to settle overnight. If natural settling is not practical, then wrap plates in plastic wrap and use gentle centrifugation.

4. Prepare schema showing which special media will be added to which wells. (Be aware that due to the need for blanks and standards for the IL-2 ELISA, not all replicates grown up will be used in the ELISA).

5. Each experiment will need 16 or 32 mL of medium with native gp120 at 5 nM/mL. FW = 120,000. Amount to add per 16 mL is 96 fL of a 100 fg/mL stock. (6 fL of 100 fg/mL for each mL of medium).

Also use 12 or 24 mL of R10 medium for each assay.

Note well: This will be the key to whether the PBMC are reactive or not. If the cells proliferate and produce IL-2 in the presence of gp120 and not without gp120, then these cells are normal reactive cells. If they behave the same vis-a-vis proliferation and IL-2 production regardless of whether gp120 has been added or not, then the cells are non-reactive. It is only the non-reactive cells that we should see the effect of IL-12 and DHEA.

6. Add 2 fg/mL of IL-2 receptor specific antibody to the 16 mL with gp120 and the 12 mL of R10 for each sample to be used for the IL-2 ELISA.

7. Preparation of DHEA:

7.1 Dissolve 1 g of DHEA in 1 mL of absolute ethanol (100%). Incubate in a 37°C water bath. Additional ethanol may be added up to 3.47 mL. This will give a 1 M solution. If the entire 3.47 mL is not needed

for it to go into solution, then the difference can be made up with R10 medium.

7.2 For each sample, we will need medium with DHEA at the following concentration: 10^{-4}, 10^{-5}, 10^{-10}, ... 10^{-12}.

7.3 At each dilution prepare 2 mL of media (already containing gp120 and antibody from step 6) and another 2 ml of media with gp120 but no antibody if a second plate is used. For 10^{-8}, 6 mL of each will be needed.

At each dilution prepare 2 x 1.5 mL of R10 without gp120 and with and without antibody from step 6. For 10^{-8}, 3 mL of each will be needed.

7.4 Making dilutions. Use 5 mL tubes.

A. Take 20 µL of 1 M DHEA into 2 mL of R10 medium = 10^{-2}M.

B. Take 20 µL of 10^{-2} M DHEA into 2 mL of step 4.3 medium = 10^{-4}M.

Take 15 µL of 10^{-2} M DHEA into 2 mL of step 4.3 medium = 10^{-5}M.

C. Take 20 µL of 10^{-4} M DHEA into 2 mL of step 4.3 medium = 10^{-6}M.

Take 15 µL of 10^{-4} M DHEA into 1.5 mL of R10 = 10^{-6}M.

D. Take 40 µL of 10^{-6} M DHEA into 4 mL of step 4.3 medium = 10^{-8}M.

Take 30 µL of 10^{-6} M DHEA into 3 mL of R10 = 10^{-8}M.

E. Take 20 µL of 10^{-8} M DHEA into 2 mL of step 4.3 medium = 10^{-10}M

Take 15 µL of 10^{-8} M DHEA into 1.5 mL of R10 = 10^{-10}M.

F. Take 20 µL of 10^{-10} M DHEA into 2 mL of step 4.3 medium = 10^{-12}M

Take 15 µL of 10^{-12}M.

7.5 To half of the 10^{-8} M DHEA media types, add antibody to human IL-12. Use at 1:1000; dilute stock 1:2, then use 3µl in 1.5 ml.

7.6 IL-12 medium for each plate:

A. To 2 ml of gp120 medium with and without antibody for IL-2, add 10 U/mL of recombinant IL-12.

B. To 1.5 mL of R10 with and without antibody to IL-2, add 10 U/mL of recombinant IL-12.

C. 1 ED_{50} = 1U. The ED_{50} of the IL-12 will be in the literature received with this reagent.

8. Aspirate medium off of cells and add 200 fL of appropriate medium to each well according to the schema. Place extra medium in peripheral wells. Wrap plates in plastic wrap and place on tray with water. Incubate at 37°C 5% CO_2.

9. If it is a two plate assay, then after 5 days aspirate off medium from the plate without antibody to the IL-2 receptor. Replace with 100 fL/well of R10 medium. Perform the cell proliferation assay with a 4 hour incubation.

10. After 7 days using the plate with the antibody to the IL-2 receptor: Take 100 fL per well and use to perform the IL-2 ELISA.

11. If there is only one plate for an assay, remove and freeze the rest of the supernatant from each well, then add 100 fL/well of R10 medium and proceed with the cell proliferation assay at this 7 day point.

12. Run PBMC from HIV- blood first to see if all reagents are performing as expected before proceeding with a HIV+ samples.

13. Another HIV- sample should be run after all the HIV+ samples have been completed.

14. Compile and analyze data.

Example 7

Administration of DHEA and Isopentenyl Adenosine 5'-Monophosphate to HIV Patients

Protocol Summary

Title: A Clinical Trial of Administered DHEA combined with isopentenyl adenosine 5'-monophosphate as a specific inhibitor of IL-10. Specifically formulated for persons with HIV infection who have developed resistance to protease and reverse transcriptase inhibitors. DHEA combined with Isopentenyl adenosine 5'-monophosphate is referred to as Compound (D+I).

Indication: Treatment of HIV-1 infection.

Type of Study: Phase I/II Clinical Trial.

Study Objectives: determine the safety and tolerance of administered D+I in persons with advanced

HIV diseases, the effect of administration of D+I on measures of HIV viral load (serum PCR (RNA) levels and HIV p24 antigen by the acid dissociation method), the immune and toxicological effects of administered D+I, and the pharmokinetics of administered D+I.

Inclusion Criteria: Age 18 years or older; HIV-1 seropositive; a CD4+ -T-lymphocyte count of 50 to 300 cells/mm^3 with one month prior to study entry, measured on two separate occasions 72 hours to 28 days apart; baseline laboratory values of Hemoglobin > 9 g/dl, WBCs > 1500 cells/μl, Neutrophils > 1000 cells/μl, Platelets 25,000 cells/μl, Bilirubin < 2.0 mg/dl, AST, ALT, Alkaline Phosphatase <5x upper limit of normal, and Creatinine < 1.5 mg/dl. In addition, a history of prior anti-retroviral therapy was required as follows: patients with a prior history of anti-retroviral therapy using AZT, dI, ddC, or d4T alone or in combination with protease inhibitors who are not receiving such therapy at study entry, must have discontinued this medication at study entry. Women of childbearing potential required one negative serum pregnancy test, beta, Δ HCG, within one week prior to study entry. A medium to high PCR HIV RNA titre was required at study entry.

Exclusion Criteria: Previous treatment with chemotherapeutic agents within eight weeks of enrollment; active, major infection, including AIDS-defining opportunistic infection, or other life-threatening medical crisis; pregnant or breast-feeding; any condition which, in the investigator's opinion places the patient

at undue risk or jeopardized the objectives of the trial; or receiving immunomodulatory therapies including interferon or pharmacological doses of steroids at entry into the study.

Safety Measures: Weekly analysis up to week 4 of the study of the following parameters: documentation and assessment of adverse events; hematology; clinical chemistries and urinalysis; assessment of the immune responses resultant from D+I; and assessment of PCR (RNA) and DNA measures alteration with therapy.

Effectiveness Measures: Measures of viral load will include HIV-p24 antigenemia, and HIV-RNA PCR (cell-free, serum) and cell HIV-DNA analysis.

Improvements in immune response will be measured as changes from baseline in CD4/CD8 ratio. Clinical lymphocyte counts, percent alterations in WBC, and percent alterations in IL-10 levels which would demonstrate the ability of D+I to cause the patients' immune system to move to T_H1 status.

Clinical benefit will be assessed by change in total body weight, Karnofsky performance score, and amelioration of signs and symptoms of disease present at baseline.

The remission or incidence of new opportunistic infection will be summarized.

Study Design: Open-label, daily administration of a dose per patient of 1200 mg/day, with review and assessment of the dosage schedules and efficacy after therapy for 4 weeks.

Study Size: 5 Patients (total) - 5 patients at 1200 mg/day for 30 days.

Test Articles: Test Drug: compound D+I with a particle size distribution in gelatine capsules of 200 mg per capsule: 87% <5 μm, 100% <15 μm. Each capsule contains: 600 mg of DHEA and 600 mg of Isopentenyl adenosine 5'-monophosphate. No control drug or placebo was used. Measurements were gathered from each patient before and after administration of D+I at weeks 0, 1, 2, 3, 4, and 8. Measurements taken were: Physical Exam & Medical History, Urinalysis, Glucose, Neopterin, beta 2-microglobulin, RBC, hB, WBC, Platelet, T Cell Panel, p24 antigen, creatinine, SGOT, SGPT, IgG, IgA, IgM, DHEA, DHEAS (DHEA sulfate),, Testosterone, 17-ketosteroids, IL-10, IL-2, PCR (RNA, cell-free, serum), PCR (DNA) and CD4. Physical examination, urinalysis, glucose, neopterin, beta 2-microglobulin, RBC, hB, p24 antigen, creatinine, IgG, IgA, and IgM were not taken at week 8. PCR (DNA) was not done at weeks 2 and 3.

To the extent not already indicated, it will be understood by those of ordinary skill in the art that any one of the various specific embodiments herein described and illustrated may be further modified to incorporate features shown in other of the specific embodiments.

The foregoing detailed description has been provided for a better understanding of the invention only and no unnecessary limitation should be understood therefrom as some modifications will be apparent to those

skilled in the art without deviating from the spirit and scope of the appended claims.

I CLAIM:

1. A method of enhancing the T_H1 immune response in a patient, comprising the step of administering to said patient:

a) an effective amount of a 17-ketosteroid compound; and

b) an effective amount of an Interleukin-10 inhibitor.

2. The method of claim 1, wherein said Interleukin-10 inhibitor is selected from the group consisting of: antiserum to Interleukin-10, a compound effective for inhibiting synthesis or biological function of Interleukin-10 and an Interleukin-10 receptor molecule blocking agent.

3. A method as recited in claim 1, wherein said Interleukin-10 inhibitor is antiserum against Interleukin-10.

4. The method of claim 3, wherein said antiserum is a monoclonal antiserum.

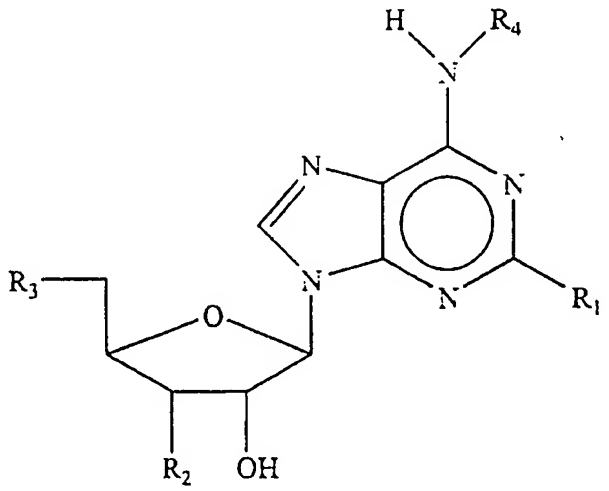
5. The method of claim 3, wherein said antiserum is an anti-murine IL-10 antiserum.

6. The method of claim 3, wherein said antiserum is an anti-human IL-10 antiserum.

7. The method of claim 3, wherein said antiserum is directed against the Epstein-Barr virus open reading frame BCRFI.

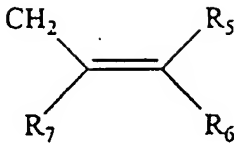
8. A method as recited in claim 1, wherein said Interleukin-10 inhibitor is selected from the group consisting of: canavanine sulfate, L-canavanine sulfate, herbimycin A, genistein, secalonic acid D, an isoflavonoid, a cytokinin, or an amphiphilic triterpenoid.

9. A method as recited in claim 1, wherein said Interleukin-10 inhibitor is selected from the group having the formula:



wherein R_1 = H, R_2 = CH_3, R_3 = CH_3, and R_4 = H

or R_4 = H or CH_3S and R_4 =

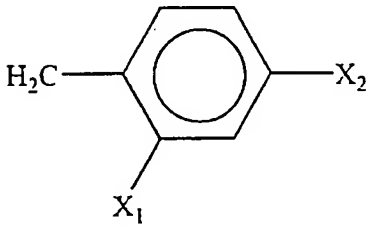


and

R_5 = CH_3, Cl, OH or a monophosphate group

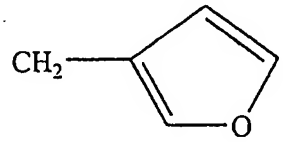
R_6 = CH_3, CH_2OH, or Cl

R_7 = H or Br or R_5 = H and R_4 =



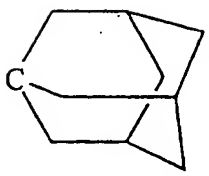
and X_1 and X_2 are independently selected from H, methyl, ethyl, hydroxyl, the halogens and carboxyl

or R_4 =

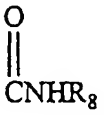


or R_4 =

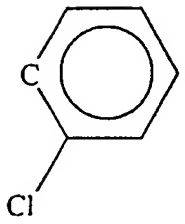
77



or R_4 =



and R_8 =

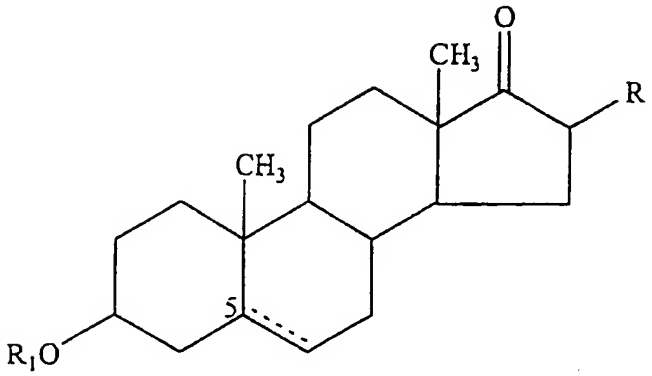


or R_8 = $(CH_2)_7CH_3$;

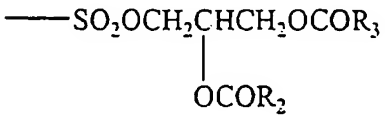
and R_2 = OH and R_3 = OH, monophosphate, diphosphate, or triphosphate group or R_2 and R_3 are linked to form a 3', 5'-cyclic monophosphate derivative.

10. The method of claim 1, wherein said Interleukin-10 inhibitor is selected from the group consisting of: N^6-(Δ^2-isopentenyl)adenine, 6-N-(3-methyl-3-hydroxybutylamino) purine, adenine, hypoxanthine, uric acid, and methylated xanthines.

11. A method as recited in any one of claims 1-10, wherein said 17-ketosteroid has the formula



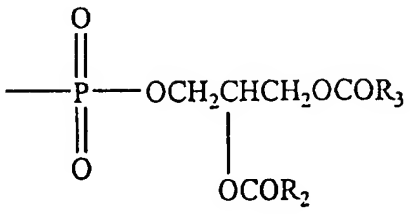
wherein R is a hydrogen atom and R_1 is a chemical group selected from the group consisting of a hydrogen atom and an SO_2OM group wherein M is selected from the group consisting of a hydrogen atom; a sodium atom; a sulphatide group



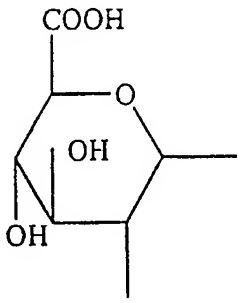
wherein each of R2 and R3, which may be the same or different, is selected from the group consisting of

straight and branched chain alkyl radicals of 1 to 14 carbon atoms;

a phosphatide group



wherein each of R2 and R3, which may be the same or different, is selected from the group consisting of straight and branched alkyl radical of 1 to 14 carbon atoms; and a glucuronide group



and wherein the atom at position 5 is present in the α- or β-configuration, or the compound comprises a mixture of both configurations.

12. A method of treating a patient for a disease, comprising the step of administering to said patient:

(1) an effective amount of a 17-ketosteroid compound; and

(2) an effective amount of an Interleukin-10 inhibitor.

13. The method of claim 12, wherein said Interleukin-10 inhibitor is selected from the group consisting of: anti-serum to Interleukin-10, a compound effective for inhibiting synthesis or biological function of Interleukin-10, and an Interleukin-10 receptor molecule blocking agent.

14. The method of claim 12, wherein said disease is a viral infection.

15. A method as recited in claim 14, wherein said disease is an HIV infection.

16. The method of claim 12, wherein said disease is a cancer.

17. A composition comprising:

(a) a 17-ketosteroid compound, and

(b) an Interleukin-10 inhibitor.

18. The composition of claim 17, wherein said Interleukin-10 inhibitor is selected from the group

consisting of: antiserum to Interleukin-10, a compound effective for inhibiting synthesis or biological function of Interleukin-10, and an Interleukin-10 receptor molecule blocking agent.

19. An article of manufacture, comprising:

(a) packaging material;

(b) at least one unit-dosage of a 17-ketosteroid compound; and

(c) a label or package insert indicating that said 17-ketosteroid compound can be used in the method of claims 1-16.

20. An article of manufacture, comprising:

(a) packaging material;

(b) at least one unit-dosage of an Interleukin-10 inhibitor; and

(c) a label or package insert indicating that said Interleukin-10 inhibitor can be used in the method of claims 1-16.

21. A method as recited in claim 1, wherein the synthesis of Interleukin-10 is inhibited by at least one cytokinin, with the proviso that said cytokinin is other than a compound of claim 9.

22. A method for preventing or reducing bacterial translocation in a patient in need of such treatment, comprising the step of administering to said patient:

(a) an effective amount of a 17-ketosteroid compound, and

(b) an effective amount of an Interleukin-10 inhibitor selected from the group consisting of: anti-serum to Interleukin-10, a compound effective for inhibiting synthesis or biological function of Interleukin-10, and an Interleukin-10 receptor molecule blocking agent.

23. A method as recited in claim 1, wherein said Interleukin-10 inhibitor is selected from the group consisting of:

(a) NG-monomethyl-1-arginine; and

(b) sodium nitroprusside.

24. A method of enhancing the T_H2 immune response in a patient, comprising the step of administering to said patient:

a) an effective amount of a 17-ketosteroid compound; and

b) an effective amount of an Interleukin-12 inhibitor.

25. The method of claim 24, wherein said Interleukin-12 inhibitor is selected from the group consisting of: antiserum to Interleukin-12, a compound effective for inhibiting synthesis or biological function of Interleukin-12 and an Interleukin-12 receptor molecule blocking agent.

26. A method as recited in claim 24, wherein said Interleukin-12 inhibitor is antiserum against Interleukin-12.

27. A method of treating a patient for a disease, comprising the step of administering to said patient:

(a) an effective amount of a 17-ketosteroid compound; and

(b) an effective amount of an Interleukin-12 inhibitor.

28. The method of claim 27, wherein said Interleukin-12 inhibitor is selected from the group consisting of: anti-serum to Interleukin-12, a compound effective for inhibiting synthesis or biological function of Interleukin-12, and an Interleukin-12 receptor molecule blocking agent.

29. The method of claim 27, wherein said disease is a bacterial infection.

30. A method of treating a patient in need of treatment for an autoimmune condition, comprising administering to said patient:

(a) an effective amount of a 17-ketosteroid compound; and

(b) an effective amount of an Interleukin-12 inhibitor.

31. The method of claim 30, wherein said autoimmune condition is lupus.

32. The method of claim 30, wherein said autoimmune condition is graft versus host disease.

33. A composition comprising:

(a) a 17-ketosteroid compound, and

(b) an Interleukin-12 inhibitor.

34. The composition of claim 33, wherein said Interleukin-12 inhibitor is selected from the group consisting of: antiserum to Interleukin-12, a compound effective for inhibiting synthesis or biological function of Interleukin-12 and an Interleukin-12 receptor molecule blocking agent.

35. The composition of claim 33, wherein said Interleukin-12 inhibitor is antiserum to Interleukin-12.

36. The composition of claim 35, wherein said antiserum is a monoclonal antiserum.

37. An article of manufacture, comprising:

(a) packaging material;

(b) at least one unit-dosage of a 17-ketosteroid compound; and

(c) a label or package insert indicating that said 17-ketosteroid compound can be used in the method of claims 24-32.

38. An article of manufacture, comprising:

(a) packaging material;

(b) at least one unit-dosage of an Interleukin-12 inhibitor; and

(c) a label or package insert indicating that said Interleukin-12 inhibitor can be used in the method of claims 24-32.

39. A method of enhancing the T_H2 immune response in a patient, comprising the step of administering to said patient:

a) an effective amount of a 17-ketosteroid compound; and

b) an effective amount of an Interleukin-2 inhibitor.

40. The method of claim 39, wherein said Interleukin-2 inhibitor is selected from the group consisting of: antiserum to Interleukin-2, a compound effective for inhibiting synthesis or biological function of Interleukin-2 and an Interleukin-2 receptor molecule blocking agent.

41. A method as recited in claim 39, wherein said Interleukin-2 inhibitor is antiserum against Interleukin-2.

42. A method of treating a patient for a disease, comprising the step of administering to said patient:

(a) an effective amount of a 17-ketosteroid compound; and

(b) an effective amount of an Interleukin-2 inhibitor.

43. The method of claim 42, wherein said Interleukin-2 inhibitor is selected from the group consisting of: antiserum to Interleukin-2, a compound effective for inhibiting synthesis or biological function of Interleukin-2, and an Interleukin-2 receptor molecule blocking agent.

44. The method of claim 42, wherein said disease is a bacterial infection.

45. A method of treating a patient in need of treatment for an autoimmune condition, comprising the step of administering to said patient:

(a) an effective amount of a 17-ketosteroid compound; and

(b) an effective amount of an Interleukin-2 inhibitor.

46. The method of claim 45, wherein said autoimmune condition is lupus.

47. The method of claim 45, wherein said autoimmune condition is graft versus host disease.

48. A composition comprising:

(a) a 17-ketosteroid compound, and

(b) an Interleukin-2 inhibitor.

49. The composition of claim 48, wherein said Interleukin-2 inhibitor is selected from the group consisting of: antiserum to Interleukin-2, a compound effective for inhibiting synthesis or biological function of Interleukin-2, and an Interleukin-2 receptor molecule blocking agent.

50. An article of manufacture, comprising:

(a) packaging material;

(b) at least one unit-dosage of a 17-ketosteroid compound; and

(c) a label or package insert indicating that said 17-ketosteroid compound can be used in the method of claims 39-47.

51. An article of manufacture, comprising:

(a) packaging material;

(b) at least one unit-dosage of an Interleukin-2 inhibitor; and

(c) a label or package insert indicating that said Interleukin-2 inhibitor can be used in the method of claims 39-47.

52. A method of enhancing the T_H1 immune response in a patient comprising the step of administering to said patient:

a) an effective amount of a 17-ketosteroid compound; and

b) an effective amount of human alpha-fetoprotein or similarly immunosuppressive peptides of human bacterial, viral or synthetic origin.

53. A method of treating a patient for an autoimmune condition, comprising the step of administering to said patient an effective amount of Interleukin-10.

54. A method of treating a patient for lupus comprising the step of administering to said patient an effective amount of Interleukin-10.

55. A method of treating a patient for graft versus host disease, comprising the step of administering to said patient an effective amount of Interleukin-10.

56. A method of treating a patient for multiple sclerosis, comprising the step of administering to said patient an effective amount of Interleukin-10.

57. A method of treating a patient receiving vaccine therapy, comprising the step of administering an effective amount of Interleukin-10 as an adjuvant to said patient.

58. A method of treating a patient in need of treatment for a disease, comprising the step of administering to said patient a combination comprising:

(a) an effective amount of Interleukin-12; and

(b) an effective amount of an Interleukin-10 inhibitor.

59. The method of claim 58, wherein said disease is cancer.

60. The method of claim 58, wherein said disease is a bacterial infection.

61. A method of enhancing endogenous Interleukin-10 levels in a patient in need of such enhancement, comprising administering to said patient an effective amount of a 17-ketosteroid compound, or an analogue or metabolite of a 17-ketosteroid compound.

62. A method as recited in claim 61, wherein said 17-ketosteroid compound is DHEA or an analogue or metabolite of DHEA.

63. A method of treating a patient in need of treatment for a disease, comprising the step of administering to said patient a combination comprising:

(a) an effective amount of a 17-ketosteroid compound; and

(b) an effective amount of a lysosomotropic agent.

64. The method of claim 63, wherein said lysosomotropic agent is selected from the group consisting of: amantadine, tributylamine, chloroquine, methylamine, quinacrine and primaquine.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 97/05716

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/565 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 355 825 A (PRENDERGAST PATRICK T) 28 February 1990 cited in the application	1-4,8-22
Y	see page 11, line 5-18; claims 1,2,9-12 --- -/--	1-52

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 1998	15.05.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kanbier, D

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INTERNATIONAL SEARCH REPORT

Inte ɔnal Application No
PCT/EP 97/05716

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 06657 A (THE GENERAL HOSPITAL CORPORATION) 9 March 1995	20, 24-28, 30, 32-34, 38-40, 42,43, 45, 47-49,51
A	see page 4, line 28-37; claim 32	1,2,12
A	see page 4, line 20-21; claims 35,37,38	13,17,21
A	see page 5, line 8-24; claims 39,41-44	57,58
A	see page 5, line 35 - page 6, line 7 see page 6-6, line 15-35; claims 45-50 see page 7, line 5-37 see page 9, line 32-37 see page 15, line 10-25	61,62

X	WO 94 08589 A (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JR UNIVERSITY) 28 April 1994	63,64
Y	see page 5, line 6-14; claims 1,6,9,11	1-52
A	see page 2, line 32-35	61,62

X	EP 0 405 980 A (SCHERING CORP) 2 January 1991	54-57
Y	see page 2, line 48 - page 3, line 12	1-52

X	WO 94 04180 A (SCHERING CORP) 3 March 1994	54-60
A	see page 1-2; claims 1-4	1-4,7, 12-14, 16-20, 39-41, 45,48-51

X	US 5 449 688 A (WAHL SHARON M ET AL) 12 September 1995	54-57
A	see column 2, line 11-25; examples 3-5,8 see column 2, line 32-44; claims 1-5 see column 3, line 51 - column 5, line 8 see column 6, line 23-42 see column 6, line 53-59	8,23

A	EP 0 340 604 A (CENTRE REGIONAL DE TRANSFUSION) 8 November 1989 see page 2, column 1-3; claims 4,5,7,8	39-42, 44-51

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1

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/EP 97/05716

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KELLOFF G J ET AL: "NEW AGENTS FOR CANCER CHEMOPREVENTION" JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 63, no. SUPPL. 26, 1996, pages 1-28, XP000676752 see page 5, right-hand column - page 6, left-hand column see page 7, right-hand column - page 8, left-hand column see page 2, right-hand column	1-4,8, 12-14, 16-20

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...national application No.
PCT/EP 97/05716

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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PCT/EP 97/05716

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Inte ɔnal Application No
PCT/EP 97/05716

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